particularly point out the claimed invention. These amendments raise no issue of new matter. Support for the amendment to claim 1 may be found in the specification, *inter alia*, on page 8, lines 5-11; page 19, line 33 - page 20, line 4. Support for "overexpresses" may be found on page 22, lines 27-35. Support for "induce cell death" may be found on page 22, line 37 to page 23, line 13. Support for the amendment to claim 11 is found on page 23, lines 29-35 of the specification. An annotated version of amended claims showing all changes relative to the previous version of that claim is attached hereto as **Exhibit A**.

Rejection Under 35 U.S.C. §112, Second Paragraph

The Examiner maintained the rejection of claims 1-5, 11, 12, and 34-37 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for the reasons of record and the reasons below.

The Examiner stated that claim 1 is vague and indefinite for the following reasons: it is unclear which cell types are suitable for evaluating neurotoxicity; it is unclear if the transfected DNA is actually expressed in the cell; and it is unclear what concentration of amyloid-beta peptide is required and which amyloid-beta peptide is required as there is no indication in the claim as to the function of the amyloid-beta peptide in the method. Thus, the Examiner took the position that it is unclear which peptide in what amount is required to practice the method.

The Examiner stated that claim 4 is rendered vague and indefinite by the phrase "a solid support" as there is no definition or examples of solid supports in the specification. Thus, the Examiner stated that it is unclear what is encompassed by a "solid support".

The Examiner stated that claims 36 and 37 are rendered vague and indefinite by the phrase "encodes for" as it is unclear how a DNA can "encode for" a protein. The Examiner stated that it is suggested that the phrase be amended to "encodes".

The Examiner stated that applicant's arguments filed 7/3/00 have been fully considered but they are not persuasive. The Examiner stated that applicants argue that with regard to "solid support", the plain language of this claim renders the claim language definite. The Examiner stated that applicants assert that a solid support is known to one of skill in the art as a material to which a compound can be affixed. The Examiner stated that Applicants provide Exhibits A-C which are references describing solid supports, published prior to the subject applications' filing date. The Examiner stated that applicants argue that the documents make clear that one of skill in the art would know the term solid support, and therefore this term is not vague. The Examiner stated that this argument is not persuasive. The Examiner stated that while "solid supports", *per se*, are well known in the art, as evidenced in Exhibits A-C, the specification does not provide any definition of the type of solid support which could be used in the method. The Examiner stated that the solid supports described in Exhibits A-C are not utilized in cell

culture systems which is required in the claim. Thus, the Examiner stated that when interpreted in light of the specification, and given the absence of any definition of "solid support" or working example of "solid support" in the method, it is not clear what type of "solid support" should be used in the method.

Applicants' Reply

In reply, applicants respectfully traverse the rejection. Claim 1 has been amended to recite a Markush group of various cells in order to specify which cells would be suitable for use in the method. Any cell which would be involved in neurotoxicity would be useful in the claimed method. Non-neuronal cell types, such as glial cells, are found in neural tissues (brain tissues) of organisms and therefore, are relevant to neurotoxicity. In addition, claim 1 has also been amended to make clear that the transfected DNA is actually expressed in the cell. The phrase "a concentration of" has been deleted from claim 1. Step (b) of claim 1 now recites: "adding amyloid-beta peptide to the cell culture to induce cell death...." The amyloid-beta peptide or fragments thereof (1-40; 1-42) are known to induce cell death. The amount of amyloid-beta peptide which is to be added is that sufficient to cause cell death, and this amount would be known to those of skill in the art.

With regard to claim 4, applicants maintain that the term "solid support" is clear and definite. It was well known at that time which solid supports were useful in cell culture. For example,

it was known that solid supports, which had attached thereto a compound, could have been added to the cell culture. See for example, Qi and Scully, (July 1997) "Extracellular collagen Modulates the Regulation of Chondrocytes by Transforming Growth Factor - Beta 1" *J. Orthop. Res.* 15(4):483-9 (attached hereto as **Exhibit 4**). This article shows that collagen type I and type II and bovine serum albumin were incorporated into alginate to form alginate beads which were then cultured with chondrocytes. In addition, a monoclonal antibody immobilized on magnetic beads has been cultured with endothelial cells (see, Saalbach, A. et al. (Dec 1997) The fibroblast-specific Mab AS02: A Novel Tool for Detection and Elimination of Human Fibroblasts. *Cell Tissue Res.* 290(3):593-9, attached hereto as **Exhibit 5**). Therefore, applicants maintain that the meaning of the term "solid support" is clear and definite in view of what was known in the art at the time.

Finally, claims 36 and 37 have been amended as suggested by the Examiner to delete the word "for."

In view of these remarks and amendments, applicants respectfully request the Examiner to reconsider and withdraw this ground of rejection.

Rejection Under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 3, 4, 11, and 12 under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for a method for evaluating the ability of a compound

to inhibit neurotoxicity comprising contacting a neuronal cell or a neuronally differentiated PC12 cell with a compound, and a pharmaceutical composition for use, *in vitro*, comprising a compound identified by the method, does not reasonably provide enablement for a method for evaluating the ability of a compound to inhibit neurotoxicity wherein the compound is a peptidomimetic, wherein the compound is bound to a solid support, or a pharmaceutical composition for use, *in vivo*.

The Examiner stated that with regard to providing a compound which is a peptidomimetic, the specification does not define what would encompass a suitable peptidomimetic, nor does not the specification identify any particular peptidomimetic or method for selecting a peptidomimetic which is suitable for use in the method for evaluating neurotoxicity. The Examiner stated that absent any guidance in the specification for selecting peptidomimetics, one of skill in the art would not have a high expectation of successfully isolating and utilizing a peptidomimetic in the claimed method without undue experimentation.

The Examiner stated that applicants' arguments filed 7/3/00 have been fully considered but they are not persuasive. The Examiner stated that applicants argue that peptidomimetics would have been fully known to one of ordinary skill in the art at the time of filing, and refers to U.S. Patent No. 5,612,895, issued March 18, 1997, (attached hereto as **Exhibit 10**) which discusses the making of peptidomimetics. The Examiner stated that applicants further argue that there are many possible "suitable" peptidomimetics

which would be encompassed by the claimed invention. The Examiner stated that these arguments are not persuasive as neither the specification nor applicants' arguments provide any particular example of a suitable peptidomimetic, nor does the specification identify any particular compound in which a peptidomimetic could be derived based on the teachings of U.S. Patent No. 5,612,895. The Examiner stated that in view of the lack of guidance in the specification as to any particular compound which is suitable in the assay, and therefore any peptidomimetic which can be derived from the compound, one of skill in the art would not have been able to make of use the peptidomimetic in the claimed method.

The Examiner stated that with regard to utilizing a compound bound to a solid support, the specification does not define what is intended by a solid support nor does the specification disclose any solid supports which are suitable for use in the claimed method. The Examiner stated that in addition, the specification does not teach the structures or classes of compounds which may be bound to solid supports. The Examiner stated that as the specification does not disclose solid supports or compounds which are suitable for attachment to a solid support, one of ordinary skill in the art would not know which supports to use for which compounds, nor would one of skill in the art have known if any and all compounds are actually capable of binding to solid supports. Thus, the Examiner stated that one of skill in the art would not have had a high expectation of successfully ascertaining which particular compound should be bound to a particular support without undue experimentation.

The Examiner stated that applicants' arguments filed 7/3/00 have been fully considered but they are not persuasive. The Examiner stated that applicants argue that with regard to "solid support", the plain language of the claim and the specification is sufficient to enable the claimed invention. The Examiner stated that Applicants assert that a solid support would have been known by one of ordinary skill to be suitable for a particular type of compound. The Examiner stated that applicants indicate, as examples, that is known that certain compounds bond well to silica type materials, other compounds bind well to plastics, and still other materials bind well to metals. The Examiner stated that these arguments are not persuasive as the specification does not provide guidance as to the type of compound which is added to the culture such that a suitable solid support, which binds to the compound can be selected. Moreover, the Examiner stated that there is no indication how the compound, bound to the solid support, is added to the culture system. The Examiner stated that while solid supports and specific compounds which bind to these supports may be well established in the art as asserted by applicants, there is no teaching in the specification, nor is there any example provided by applicants' arguments which indicate which compound and appropriate solid support to select, and how to add the compound bound to the solid support to the culture system. The Examiner stated that the specification does not provide any guidance as to how to make or use the compound/solid support element of the claimed invention.

The Examiner stated that, as written, the pharmaceutical

composition of claims 11 and 12 encompasses both *in vitro* and *in vivo* applications. Moreover, the Examiner stated that the compound can encompass macromolecules such as nucleic acids.

The Examiner stated that while the specification is enabling for providing a pharmaceutical composition to cells *in vitro* to establish whether the compound inhibits neurotoxicity, the specification is non-enabling for administering a pharmaceutical composition which inhibits neurotoxicity *in vivo*. The Examiner stated that the specification does not provide any correlation with respect to the *in vitro* and *in vivo* effectiveness of a compound as the inhibition of neurotoxicity has only been demonstrated *in vitro*.

The Examiner stated that the specification does not disclose which compound would be suitable for treating a particular disease, whether the same compound would successfully treat all recited diseases, what dosages and routes of administration of the compound would be effective in treating a specific disclosed disease, and how one would ascertain whether the pharmaceutical composition was effective in ameliorating a specific disclosed disease. In addition, the Examiner stated that the state of the art at the time of filing indicates that treating neurodegenerative diseases is neither routine nor predictable.

The Examiner stated that the specification does not provide any compound which is characterized such that one of skill in the art would be able to determine its mechanism of action, *in vivo*

turnover rates, the mode of administration, the amount to administer and the frequency of administration.

The Examiner stated that with regard to the Sabate et al. reference which was relied upon in the office action of 1/3/00 to provide evidence that treating neurodegenerative diseases is neither routine nor predictable, applicants argue that the blood brain barrier is merely one challenge in optimizing administration of an inhibitor of neurotoxicity, e.g., intracranial implants or injections are possible modes of administration which could be successful. The Examiner stated that this argument is not persuasive as applicants are providing hypothetical examples of possible modes which could be successful, which are not described in the specification. The Examiner stated that applicants also point out that there are no claims pending to "treatment of neurodegenerative disease", however, the pharmaceutical composition recited in the claims are fully enabled by the subject specification. The Examiner stated that while the examiner agrees that there are no treatment method claims, as indicated in the office action of 1/3/00, the intended use of the pharmaceutical composition is for treatment of a myriad of neurodegenerative diseases and disorders. The Examiner stated that as the specification does not provide any example of a compound obtained by a process which could be incorporated into a pharmaceutical composition and which can be used in a treatment method for any one of the disclosed neurodegenerative diseases or disorders, one of skill in the art would not be able to make or use the pharmaceutical composition as intended in a predictable and reproducible manner and without undue

experimentation. Thus, the Examiner stated that while the specification is enabling for a pharmaceutical composition which can be used *in vitro* applications, the specification is not enabling for a pharmaceutical composition which can be used in *in vivo* applications.

The Examiner stated that with regard to applicants argument that the method is enabled for use in vivo, applicants assert that the cells transfected with DNA encoding RAGE and mutant PS-2 could be part of the organism and that the claimed method could be carried out in vivo by routine methods. The Examiner stated that Applicants suggest that one of ordinary skill in the art could make a transgenic mouse which has been engineered with a DNA construct which encodes RAGE and a mutant PS-2 and the mouse could be administered a compound and amyloid-beta peptide, and subsequently analyzed for apoptotic activity. The Examiner stated that this argument is not persuasive as the specification does not disclose how to make transgenic mice comprising a heterologous RAGE and a mutant PS-2, nor does the specification teach a particular phenotype displayed by the transgenic mice. Moreover, the Examiner stated that the generation of transgenic mice which display a particular phenotype is neither routine nor predictable. For example, the Examiner stated that Palmiter et al. (Proc. Natl. Acad. Sci, USA 1991) teach that directed expression of any gene to any specific cell type of an animal by using established transgenic methodology is theoretically possible by combining the regulatory region(s) of a gene that is expressed in a cell-specific manner with any mRNA-encoding structural gene. The Examiner stated that Palmiter et al. note,

however, that not all gene constructs work well; the two most common problems are inappropriate expression patterns and failure to achieve adequate expression levels (see page 478, left column, first paragraph). The Examiner stated that Kappel et al. (Current Opinion in Biotechnology, 3:548-553, 1992) teach that while transgenes can be targeted, inherent cellular mechanisms may alter the pattern of gene expression (see, e.g., page 549, right column). In addition, the Examiner stated that Cameron (Molecular Biology, 7:253-265, 1997) teaches that "[w]ell-regulated transgene expression is the key to successful transgenic work, but all too often experiments are blighted by poor levels or the complete absence of expression, as well as less common problems, such as leaky expression in nontargeted tissues...". "A feature common to many transgenic experiments is the unpredictable nature of transgene expression with different lines produced with the same construct frequently displaying different levels of expression. Further, expression levels do not correlate with the number of transgene copies integrated...". "Such copy-number-independent, intergration-site-dependent expression patterns emphasize the influence of surrounding chromatin on the transgene" (see, e.g., page 256 under "Transgene Regulation and Expression").

The Examiner stated that as the specification does not disclose how to make such a transgenic mouse, and view of the unpredictability of generating a transgenic mouse with a particular phenotype as indicated in the state of the art of transgenics, applicants' reliance on a hypothetical example of providing the pharmaceutical composition to a non-disclosed

transgenic mouse is not persuasive.

Applicants' Reply - Claimed Invention is Fully Enabled

In reply, applicants respectfully traverse the rejection and maintain that the claimed invention is fully enabled by the subject specification. One of skill in the art would have been able to make and use the claimed invention in view of the subject specification combined with what one of skill in the art would have known as of the filing date, December 23, 1997, without undue experimentation.

Applicants' claimed invention is directed, *inter alia*, to a method for for evaluating the ability of a compound to inhibit neurotoxicity which comprises: (a) contacting a cell which overexpresses (i) a receptor for advanced glycation end product (RAGE) protein and (ii) a mutant presenilin-2 protein with the compound, wherein the cell is selected from the group consisting of a neuronal cell, an endothelial cell, a glial cell, a microglial cell, an astrocyte, a neuronal tumor cell, a PC12 cell, a mononuclear cell, a mononuclear phagocyte, a smooth muscle cell, a bone marrow cell and a myocyte, and wherein the mutant presenilin-2 protein causes increased basal apoptosis in nerve growth factor-differentiated PC12 cells; (b) adding amyloid-beta peptide to the cell culture to induce cell death; (c) determining the level of cell death in the cell culture; and (d) comparing the level of cell death determined in step (c) with the amount determined in the absence of the compound so as to

evaluate the ability of the compound to inhibit neurotoxicity. The pending claims are also directed to pharmaceutical compositions.

Peptidomimetics

The Examiner is concerned that the pending claims are not enabled for isolating and utilizing a peptidomimetic without undue experimentation. Applicants first point out that enablement of claims does not turn on whether a working example is disclosed in the specification. Indeed, the M.P.E.P. § 2164.02 recites that "an applicant need not have actually reduced an invention to practice prior to filing." In addition, the enablement requirement is determined in view of a combination of what was known at the time to one of skill in the art and the disclosure of the instant application. In this case, it was known to one of skill in the art how to make a peptidomimetic, if provided with a peptide.

One of skill in the art as of December 23, 1997 would have known how to make peptidomimetics using rational drug design. As evidence of this, applicants attach hereto as **Exhibit 11,** U.S. Patent No. 5,331,573 which is entitled "Method of Design of Compounds That Mimic Conformational Features of Selected Peptides," issued July 19, 1994. The Abstract states that "rational design of novel compounds, useful as drugs, e.g., bioactive peptidomimetic compounds...is thus made possible using the simulation methods and tools of the described invention." U.S. Patent No. 5,612,895, is entitled "Method of Rational Drug

Design Based on Ab Initio Computer Simulation of Conformational Features of Peptides" and a copy is attached hereto as **Exhibit 10**. U.S. Patent No. 5,552,534, issued September 3, 1996, is entitled "Non-peptide Peptidomimetics" and a copy is attached hereto has **Exhibit 12**. Applicants submit that one of skill in the art would have known as of the effective filing date, December 23, 1997, how to make and use peptidomimetics in view of the teachings of the aforementioned references combined with the disclosure of the subject application which teaches how to determine whether a given peptide is able to inhibit neurotoxicity. The subject specification provides methods for identifying a peptide which inhibits neurotoxicity. One example of a peptide is a soluble extracellular portion of RAGE, see page 9, lines 6-7 of the specification.

The subject specification provides an example of a compound which is a peptide, e.g., soluble RAGE. In addition, the sequence of RAGE was known and published as of the effective filing date, December 23, 1997, see Yan et al., 1996 (Exhibit 24 of IDS, previously filed in connection with the instant application on November 10, 1998). Therefore, the <u>combination</u> of what one of skill in the art would have known as of December 23, 1997 <u>and</u> the disclosure of the subject application is sufficient to enable one of skill in the art to make and use the claimed invention without undue experimentation. One of skill in the art is taught by the specification to carry out the method recited in claim 1 to determine whether a peptide is able to inhibit neurotoxicity and that peptide could be the basis for obtaining a peptidomimetic using the known methods described in the earlier patents and

references mentioned hereinabove. Therefore, applicants request the Examiner to reconsider and withdraw this ground of rejection.

Solid Support

Claim 4 recites "wherein the compound is bound to a solid support." This claim is fully enabled by the subject specification combined with what one of skill in the art would have known as of the filing date. Applicants again point out that enablement of claims does not turn on whether a working example is disclosed in the specification. Indeed, the M.P.E.P. § 2164.02 recites that "an applicant need not have actually reduced an invention to practice prior to filing. In addition, the enablement requirement is determined in view of a combination of what was known at the time to one of skill in the art and the disclosure of the instant application. In this case, it was known to one of skill in the art how to use a solid support in a cell culture. For example, it was known that solid supports, which had attached thereto a compound, could have been added to the cell culture. See for example, Qi and Scully, (July 1997) "Extracellular collagen Modulates the Regulation of Chondrocytes by Transforming Growth Factor - Beta 1" *J. Orthop. Res.* 15(4):483-9 (attached hereto as **Exhibit 4**). This article shows that collagen type I and type II and bovine serum albumin were incorporated into alginate to form alginate beads which were then cultured with chondrocytes. In addition, a monoclonal antibody immobilized on magnetic beads has been cultured with endothelial cells (see, Saalbach, A. et al. (Dec 1997) The fibroblast-

specific Mab AS02: A Novel Tool for Detection and Elimination of Human Fibroblasts. *Cell Tissue Res.* 290(3):593-9, attached hereto as **Exhibit 5**). Attachment of compounds to magnetic beads, or alginate, or other solid supports was well known in the art at the time of filing.

Therefore, applicants submit that the claimed invention as to peptidomimetics was fully enabled as of the filing date, December 23, 1997. Applicants request the Examiner to reconsider and withdraw this ground of rejection.

In vivo use of method

The Examiner acknowledges that the claimed method is enabled for use *in vitro*, but is concerned that the claimed method is not enabled for use *in vivo*. Applicants maintain that one of ordinary skill in the art would be fully enabled by the subject specification to carry out the claimed method *in vivo*. For example, the claimed method as recited in claim 1 calls for a cell which expresses RAGE and mutant PS-2. Applicants emphasize that this cell could be part of an organism and that the claimed method could be carried out *in vivo* by routine methods. For example, one of ordinary skill would be able to make, by routine methods, a transgenic mouse which has been engineered with a DNA construct which encodes RAGE and a mutant PS-2. The mouse could then be administered a test compound so that the compound contacts a cell within the mouse and then the mouse could be administered a concentration of amyloid-beta peptide. This

administration could be carried out via perfusion of the brain, cranial injection, etc. (all routine methods). Finally, the mouse could be sacrificed and the cells be surveyed for cell death (by histological staining, by microscopy, etc.) or alternatively, a non-invasive method could be utilized to determine the level of cell death. Clearly, the application of the claimed method to *in vivo* use would be routine to one of ordinary skill in the art.

Transgenic mouse models of Alzheimer's Disease were established in the art as of December 23, 1997. Indeed, several transgenic mouse models expressed mutant forms of presenilins. For example, see Citron et al. (Jan 1997) "Mutant Presenilins of Alzheimer's Disease Increase Production of 42-residue Amyloid Beta-protein in Both tranfected Cells and Transgenic Mice" *Nature Medicine*, 1:67-72 (attached hereto as **Exhibit 6**). A transgenic mouse model was established that co-expresses human presenilin (wild-type and mutant) and amyloid beta-protein precursor genes. In addition, see Mattson and Guo (Nov 1997) "Cell and Molecular Neurobiology of Presenilins: A Role For The Endoplasmic Reticulum In The Pathogenesis of Alzheimer's Disease?" *J. Neurosci. Res.* 50(4):505-13 (attached hereto as **Exhibit 7**). This paper shows expression of mutant presenilins in transgenic mice. In addition, Borchelt et al. (October 1997) "Accelerated Amyloid Deposition In the Brains of Transgenic Mice Coexpressing Mutant Presenilin 1 and Amyloid Precursor Proteins." *Neuron* (October 1997) 19(4):939-45 (attached hereto as **Exhibit 8**) report the making of transgenic animals that express a human PS1 variant.

Finally, the Examiner's attention is directed to Lamb et al. (September 1997) "Altered Metabolism of familial Alzheimer's Disease-linked Amyloid Precursor Protein Variants in Yeast Artificial Chromosome Transgenic Mice." *Hum. Mol. Genet.* 6(9):1535-41 (attached hereto as **Exhibit 9**). These papers are a sample of the reports which exist prior to December 23, 1997 of transgenic animals created as models of Alzheimer's Disease which support the applicants position that the making of a transgenic mouse coexpressing mutant presenilin-2 and RAGE would not have required undue experimentation by one of ordinary skill in the art.

Applicants' specification in combination with what was known to one of skill in the art as of the effective filing date (i.e., December 23, 1997) would have enabled the skilled person to carry out the presently claimed invention without undue experimentation. The rejection should therefore be reconsidered and withdrawn.

Rejection Under 35 U.S.C. §102(b) - Bartus et al.

The Examiner rejected claims 11 and 12 under 35 U.S.C. §102(b) as being anticipated by Bartus et al. (U.S. Patent No. 5,444,042, 1995) for the reasons of record, and the reason below.

The Examiner stated that Bartus et al. teach compounds which inhibit neurotoxicity, i.e., calpain inhibitors. The Examiner stated that the calpain inhibitors effectively block cell death in an *in vitro* model for neuropathology (see column 73, lines 5-

24). The Examiner stated that compounds can be formulated as pharmaceutical compositions comprising the compound of interest in a pharmaceutically acceptable formulation containing a carrier material (see column 4, lines 48-54 and column 66, lines 36-40).

The Examiner stated that the claimed pharmaceutical composition is obtained by a particular process. However, The Examiner stated that the process does not provide any structural characteristics of the composition. The Examiner stated that the only functional limitation of the composition is that inhibits neurotoxicity. In this regard, the Examiner stated that the pharmaceutical composition of Bartus et al. also inhibits neurotoxicity. Thus, the neurotoxicity inhibitor of Bartus et al. anticipates the claimed neurotoxicity inhibitor.

In reply, applicants traverse the rejection. Claim 11 has been amended to recite "a pharmaceutical composition which comprises a compound which inhibits neurotoxicity in a cell by inhibiting interaction between receptor for advanced glycation endproduct and mutant presenilin-2 identified by the method of claim 1, and a pharmaceutically acceptable carrier." The calpain inhibitor of Bartus et al. does not inhibit neurotoxicity by inhibiting an interaction between RAGE and mutant presenilin-2. The cell death which is inhibited by the calpain inhibitors is glutamate-induced. The compounds which are identified by the method of claim 1 (as recited in claim 11) are compounds which inhibit cell death induced by amyloid-beta peptide, not induced by glutamate. In addition, Bartus et al. do not teach that the compounds which inhibits neurotoxicity in a cell by inhibiting interaction

between receptor for advanced glycation endproduct and mutant presenilin-2 as required by amended claim 11. Bartus et al. merely state that "calpain inhibitors are neuroprotective in vivo" (73:42-22). There is no teaching of the pharmaceutical compositions claimed because there is no disclosure of inhibition of an interaction between RAGE and mutant presenilin-2 by calpain inhibitors by Bartus et al.

For the foregoing reasons, applicants maintain that Bartus et al. do not anticipate the claimed invention. Applicants request that the Examiner reconsider and withdraw this ground of rejection.

Rejection Under 35 U.S.C. §103(a)

The Examiner rejected claims 1-5, 11, 12, and 34-37 under 35 U.S.C. §103(a) as being unpatentable over Wolozin et al. (Science, 274:1710-1713, December 6, 1996) taken with Yan et al. (Nature, 382:685-691, 1996, newly applied).

The Examiner stated that Wolozin et al. disclose that transfecting neuronally differentiated PC12 cells with a mutant presenilin-2 protein (e.g., N141I) causes increased basal apoptosis in nerve growth factor-differentiated PC12 cells (see, e.g., page 1711, left column, middle column, and Figure 1). In addition, the Examiner stated that Wolozin et al. disclose a method comprising a)culturing the neuronally differentiated PC12 cells in the presence or absence of a compound, i.e., pertussis toxin or Aâ, (1-42), b)determining the level of apoptosis in the control and treated cells, and c) comparing the extent of

apoptotic activity in the cells cultured in the presence of the compound compared to cells cultured in the absence of the compound to evaluate the effect of the compound on apoptotic activity (see, e.g., page 1711, middle and right columns, page 1712, left column, Figure 3, and Figure 4E). The Examiner stated that the Aâ(1-42) compound is added to the cells at a concentration of 10μM and was generated from a 1mM Aâ(1-42) stock solution (see, e.g., page 1713, Note #21). Thus, the Examiner stated that Wolozin et al. disclose the claimed method and pharmaceutical composition comprising a compound and a pharmaceutical carrier. The Examiner stated that while Wolozin et al. do not disclose adding a nucleic acid compound to a neuronally differentiated PC12 cells expressing a mutant presenilin-2 protein, or all of the claim-designated pharmaceutical carriers, Wolozin et al. does not disclose adding PS-2 or ALG-3 antisense nucleic acids to neuronally differentiated PC12 cells which do not express a mutant presenelin-2 protein. The Examiner stated that addition of the antisense nucleic acid results in a decrease in apoptotic activity in the PC12 cells (see, e.g., page 1720, middle and right columns, and Figure 1). The Examiner stated that inasmuch as Wolozin et al. disclose that PC12 cells that express a mutant presenilin-2 protein have a high apoptotic activity, it would have been obvious to add PS-2 or ALG-3 antisense nucleic acids to neuronally differentiated PC12 cells expressing mutant presenilin-2 to determine if the antisense nucleic acids are effective in decreasing the observed apoptotic activity in PC12 cells expressing mutant presenilin-2 protein. Moreover, adding the nucleic acids, or other compounds such as pertussis toxin or

Aâ(1-42) to cell cultures as a pharmaceutical composition would have been obvious and well within the purview of one of ordinary skill in the art of cell culture. The Examiner stated that one of ordinary skill in the art would have been motivated to admix the compound added to the cell culture and to avoid a localized high concentration of a solid compound which may be detrimental to the cells.

The Examiner stated that Wolozin et al. do not teach that the PC12 cells are transfected with a DNA sequence encoding RAGE and which is expressed in PC12 cells. However, the Examiner stated that Yan et al. teach that enhanced expression of RAGE in Alzheimer's diseases, in affected neurons, in microglia and in vasculature, is consisting with the concept that Aâ-RAGE interaction may contribute to neurotoxicity that results in dementia (see page 382, left column, last paragraph). Thus, the Examiner stated that it would have been obvious to one of ordinary skill in the art to provide cells associated with neurodegenerative diseases. The Examiner stated that Yan et al. further teach that human Aâ(1-40) or Aâ(1-42) purified from plaques or vascular amyloid from Alzheimer's disease patients inhibits binding of Aâ to RAGE (see e.g., page 688, left column); that Aâ binding to RAGE and Aâ0-induced cellular perturbation results in oxidant stress and cytotoxicity (see, e.g., page 688, right column, under "RAGE and Aâ-induced cellular stress"). The Examiner stated that Yan et al. indicate that RAGE can mediate Aâ-induced oxidant stress on endothelium and neuronal cells and that the stress can be prevented by blocking access to RAGE using either anti-RAGE IgG or excess soluble receptor, and further

teach that expression of RAGE increases vulnerability to Aâ. The Examiner stated that Yan et al. indicate that RAGE, if present and/or upregulated in cells important in the pathogenesis of Alzheimer's disease, could mediate toxic effects when associated with Aâ. The Examiner stated that note also that Yan et al. teach transfection of RAGE into COS-1 cells and the use of these transfected cells in analyzing the effect of compounds on Aâ activity with respect to oxidant stress (see, page 688, under "RAGE and Aâ-induced cellular stress").

The Examiner stated that it would have been obvious to one of ordianry skill in the art at the time the claimed invention was made to modify the method of Wolozin et al. by further modifying the presenilin-2 transfected PC12 cells of Wolozin et al. by transfecting the cells with a vector encoding RAGE in veiw of the teachings of Yan et al. that cells transfected with RAGE are useful in studying the interaction of RAGE and Aâ on oxidant stress and cytotoxicity in cells. The Examiner stated that one of skill in the art would have been motivated to provide such a modified PC12 cell to use in a method of identifying inhibitors of neurotoxic compounds, such as those associated with Alzheimer's disease, in view of the teachings of Yan et al. that enhanced expression of RAGE in Alzheimer's disease, in affected neurons, in microglia and in vasculature, is consisting with the concept that Aâ-RAGE interaction may contribute to neurotoxicity that results in dementia.

Thus, the Examiner stated that the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention

was made especially in the absence of sufficient, clear, and convincing evidence to the contrary.

In reply, applicants traverse the rejection and submit that the combination of Wolozin et al. and Yan et al. do not make obvious the claimed invention.

The claimed invention is directed to a method for evaluating the ability of a compound to inhibit neurotoxicity which comprises: (a) contacting a cell which overexpresses (i) a receptor for advanced glycation end product (RAGE) protein and (ii) a mutant presenilin-2 protein with the compound, wherein the cell is selected from the group consisting of a neuronal cell, an endothelial cell, a glial cell, a microglial cell, an astrocyte, a neuronal tumor cell, a PC12 cell, a mononuclear cell, a mononuclear phagocyte, a smooth muscle cell, a bone marrow cell and a myocyte, and wherein the mutant presenilin-2 protein causes increased basal apoptosis in nerve growth factor-differentiated PC12 cells; (b) adding amyloid-beta peptide to the cell culture to induce cell death; (c) determining the level of cell death in the cell culture; and (d) comparing the level of cell death determined in step (c) with the amount determined in the absence of the compound so as to evaluate the ability of the compound to inhibit neurotoxicity.

First, applicants submit that there is no motivation for one of ordinary skill in the art to combine Wolozin et al. and Yan et al. and even if there was motivation, the combination does not make the claimed invention obvious.

The only common thread between the two references is that each paper characterizes a molecule which is involved in neurotoxicity or neurodegeneration in Alzheimer's disease. Wolozin et al. disclose overexpression of presenilin-2 in PC12 cells as causing increased apoptosis induced by trophic factor withdrawal or beta-amyloid (see Abstract). Yan et al. disclose the isolation and characterization of a polypeptide which binds amyloid-beta peptide, i.e. RAGE (see p 685, column 1). Wolozin et al. do not disclose RAGE or suggest that presenilin-2 may interact with RAGE. Yan et al. do not disclose or suggest that RAGE may interact with presenilin-2. The Examiner has not given a reason why one of ordinary skill in the art would have been motivated to combine these references. The Examiner merely states that "it would have been obvious to one of ordinary skill in the art to provide cells associated with neurodegenerative diseases..." (see Office Action, page 12). Applicants request the Examiner to consider a recent Federal Circuit decision, In re Rouffet, 47 USPQ2d 1453 (Fed. Cir. 1998) which states:

> To prevent the use of hindsight, this court requires the examiner to show a motivation to combine the references that create the obviousness. In other words, the examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select elements from the cited prior art for combination in the manner claimed.

Applicants submit that the Examiner is using impermissible hindsight in combining the cited references. The Federal Circuit has stated that

> rejecting patents solely by finding prior art corollaries for the claimed elements would permit an examiner to use the claimed invention itself as a blueprint for piecing together elements in the prior art to defeat the patentability of the claimed invention. Such an approach would be "an illogical and inappropriate process by which to determine patentability.

Sensonics, Inc. v. Aerosonic Corp., 81 F.3d 1566, 1570, 38 USPQ2d 1551, 1554 (Fed. Cir. 1996). In addition, the Federal Circuit has held that it will infer the use of hindsight in the selection of references that comprise the case of obviousness without an explanation of the specific understanding or principle within the knowledge of one of ordinary skill in the art that would motivate one with no knowledge of the claimed invention to make the combination. See *In re Gorman*, 933 F.2d 982, 986, 18 USPQ2d 1885, 1888 (Fed. Cir. 1991). Finally, the Federal Circuit stated that if such "a rote invocation [of high level of skill in the art] could suffice to supply a motivation to combine, the more sophisticated scientific fields would rarely, if ever, experience a patentable technical advance." See *In re Rouffet*, 47 USPQ2d 1453, 1458 (Fed. Cir. 1998). Applicants believe that the Examiner the Examiner has used hindsight analysis to identify the two cited references and combine them because one of ordinary skill in the art without knowing applicants' invention, would have no motivation or suggestion to combine them.

Notwithstanding the above discussion, applicants submit that the combination of Wolozin et al. and Yan et al. do not render obvious the claimed invention. Wolozin et al. do not teach or suggest cells which overexpress a mutant presenilin-2 protein. There is no hint or suggestion in Wolozin et al. that presenilin-2 is involved in any way with RAGE receptor proteins. Therefore, there is no suggestion to one of ordinary skill to carry out step (a) of the claimed invention.

Contrary to the Examiner's assertion, Wolozin et al. do not "disclose the claimed method" (see Office Action, page 11, last four lines). The steps of claim 1 are not disclosed. For example, Wolozin et al. do not teach or suggest the cell overexpressing RAGE and presenilin-2 as recited in claim 1. The claimed invention specifically recites that the pharmaceutical composition comprises a compound "which inhibits neurotoxicity in a cell by inhibiting interaction between receptor for advanced glycation endproduct and mutant presenilin-2." The Abstract of Wolozin, et al. states that apoptosis induced by PS2 protein was sensitive to pertussis toxin, "suggesting that heterotrimeric GTP-binding proteins are involved." There is no teaching to make obvious the pharmaceutical compositions claimed.

The Examiner states that "it would have been obvious to add PS-2 or ALG-3 antisense nucleic acids to neuronally differentiated PC12 cells expressing mutant presenilin-2 to determine if the antisense nucleic acids are effective in decreasing the observed apoptotic activity in PC12 cells expressing mutant presenilin-2

protein." Applicants emphasize that the addition of such antisense molecules disclosed in Wolozin et al. does not render obvious the claimed invention. The Examiner is correlating the antisense molecules with the compound recited in claim 1. However, "neuronally differentiated PC12 cells expressing mutant presenilin-2" do not make obvious the cells recited in step (a) of claim 1 which overexpress RAGE and mutant PS-2. There is no recognition in Wolozin et al. of the existence of or significance of an interaction between RAGE and PS-2.

Furthermore, the combination of Wolozin et al. and Yan et al. do not render obvious the pharmaceutical compositions presently claimed which comprise a compound which inhibits neurotoxicity in a cell by inhibiting interaction between receptor for advanced glycation endproduct and mutant presenilin-2. See amended claim 11. The interaction between RAGE and presenilin-2 is not taught in either Wolozin et al. or Yan et al. The Examiner has used impermissible hindsight to combine these two references.

In view of these remarks, applicants maintain that Wolozin et al. in combination with Yan et al. do not render obvious the claimed invention. Applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Supplemental Information Disclosure Statement

In accordance with their duty of disclosure under 37 C.F.R. §1.56 and §1.97 (a)-(b), applicants would like to direct the Examiner's attention to the following documents:

1. Kochi, S. et al. Life Science 2000, 66(23):2255-60 **(Exhibit 2)**;

2. Hirase, T. et al. J. Cell Sci (Jul 1997) 110(Pt 4):1603-13. **(Exhibit 3)**;

3. Qi and Scully, (July 1997) "Extracellular collagen Modulates the Regulation of Chondrocytes by Transforming Growth Factor - Beta 1" *J. Orthop. Res.* 15(4):483-9 (**Exhibit 4**);

4. Saalbach, A. et al. (Dec 1997) The fibroblast-specific Mab AS02: A Novel Tool for Detection and Elimination of Human Fibroblasts. *Cell Tissue Res.* 290(3):593-9, (**Exhibit 5**);

5. Citron et al. (Jan 1997) "Mutant Presenilins of Alzheimer's Disease Increase Production of 42-residue Amyloid Beta-protein in Both tranfected Cells and Transgenic Mice" *Nature Medicine*, 1:67-72 (**Exhibit 6**);

6. Mattson and Guo (Nov 1997) "Cell and Molecular Neurobiology of Presenilins: A Role For The Endoplasmic Reticulum In The Pathogenesis of Alzheimer's Disease?" *J. Neurosci. Res.* 50(4):505-13 (**Exhibit 7**);

7. Borchelt et al. (October 1997) "Accelerated Amyloid Deposition In the Brains of Transgenic Mice Coexpressing Mutant Presenilin 1 and Amyloid Precursor Proteins." *Neuron*

(October 1997) 19(4):939-45 (**Exhibit 8**);

8. Lamb et al. (September 1997) "Altered Metabolism of familial Alzheimer's Disease-linked Amyloid Precursor Protein Variants in Yeast Artificial Chromosome Transgenic Mice." *Hum. Mol. Genet.* 6(9):1535-41 (**Exhibit 9**);

9. U.S. Patent No. 5,612,895, Method of Rational Drug Design Based on Ab Initio Computer Simulation of Conformational Features of Peptides, issued March 18, 1997 (**Exhibit 10**);

10. U.S. Patent No. 5,331,573 "Method of Design of Compounds That Mimic Conformational Features of Selected Peptides," issued July 19, 1994 (**Exhibit 11**); and

11. U.S. Patent No. 5,552,534, Non-peptide Peptidomimetics, issued September 3, 1996, (**Exhibit 12**).

The above references are again listed on the substitute PTO Form 1449 attached hereto as **Exhibit 1.** Copies of the above-listed references are attached hereto as **Exhibits 2-12**. The Information Disclosure Statement fee of $180.00 is enclosed herewith. Applicants request that the Examiner make these documents of record in the subject application.

If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone at the number provided below.

No fee other than the $445.00 three-month extension of time fee and the $180.00 Information Disclosure Statement fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

Jane M. Love

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Jane M. Love 4/4/01

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